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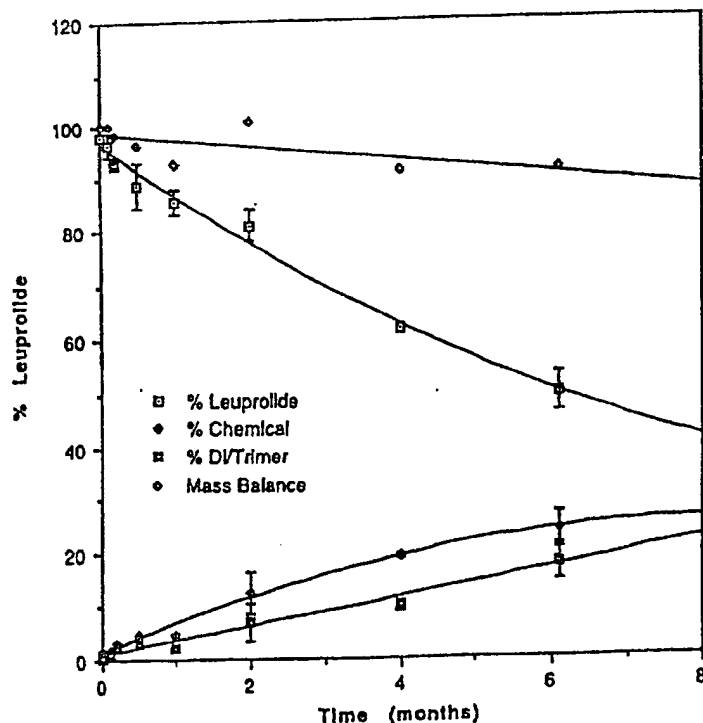
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(54) Title: **NON-AQUEOUS POLAR APROTIC PEPTIDE FORMULATIONS**

(57) Abstract

This invention relates to stable non-aqueous polar aprotic formulations of peptide compounds. These stable formulations comprise peptide in non-aqueous polar aprotic solvent. They may be stored at elevated temperatures for long periods of time and are especially useful in implantable delivery devices for long term delivery of drug.



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1
2 **NON-AQUEOUS POLAR APROTIC PEPTIDE FORMULATIONS**

3
4 **CROSS-REFERENCE TO RELATED APPLICATIONS**

5 This application claims priority under 35 U.S.C. 119(e) to U.S.
6 Application Serial No. 60/022,699 filed July 3, 1996, the disclosure of which is
7 incorporated herein by reference.

8
9 **FIELD OF THE INVENTION**

10 This invention relates to stable non-aqueous polar aprotic formulations
11 of peptide compounds and more particularly to formulations of peptide
12 compounds at high concentrations.

13
14 **BACKGROUND OF THE INVENTION**

15 References:

16 The following references are referred to by numbers in brackets ([]) at
17 the relevant portion of the specification.

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16 *Research*, 11/8, pages 1143-1147 (1994).

17 The disclosure of each of the above publications, patents or patent
18 applications is hereby incorporated by reference in its entirety to the same
19 extent as if the language of each individual publication, patent and patent
20 application were specifically and individually incorporated by reference.

21 Luteinizing hormone-releasing hormone (LHRH), also known as
22 gonadotropin releasing hormone (GnRH), is a decapeptide with the structure:
23 pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂.

24 It is secreted by the hypothalamus and binds to receptors on the pituitary
25 gland, releasing luteinizing hormone (LH) and follicle stimulating hormone
26 (FSH). LH and FSH stimulate the gonads to synthesize steroid hormones.
27 Numerous analogs of LHRH are known, including peptides related to LHRH
28 which act as agonists and those which act as antagonists. [1-15] LHRH
29 analogs are known to be useful for treating hormone-dependent diseases
30 such as prostate cancer, benign prostatomegaly, endometriosis,
31 hysteromyoma, metrofibroma, precocious puberty, or mammary cancer and
32 as contraceptives. [8] Sustained release administration is preferred for both

1 agonist LHRH-related compounds, which reduce the number of available
2 receptors after repeated administration so that the production of steroid
3 hormones is suppressed, and antagonist LHRH-related compounds, which
4 must be continually administered for persistent inhibition of endogenous
5 LHRH. [8]

6 The sustained parenteral delivery of drugs, especially peptide drugs,
7 provides many advantages. The use of implantable devices for sustained
8 delivery of a wide variety of drugs or other beneficial agents is well known in
9 the art. Typical devices are described, for example, in U.S. Patents Nos.
10 5,034,229; 5,057,318; and 5,110,596. The disclosure of each of these
11 patents is incorporated herein by reference.

12 In general, oral bioavailability of peptides, including LHRH-related
13 compounds, is low. [16-17]

14 Currently marketed formulations of LHRH, its analogs and related
15 compounds which are used for parenteral injection are aqueous solutions
16 which contain relatively low concentrations of LHRH-related compounds (0.05
17 to 5 mg/ml) and may also contain excipients such as mannitol or lactose. [18-
18 20] Such formulations of LHRH-related compounds must either be stored
19 refrigerated or may be stored at room temperature for short periods of time.

20 Available depot formulations of LHRH-related compounds
21 administered for sustained release over a period of 1-3 months include a
22 formulation comprised of 15% LHRH-related compound dispersed in a matrix
23 of D,L-lactic and glycolic acids copolymer presented as a cylinder to be
24 injected subcutaneously [1] and a formulation comprised of microparticles
25 comprising a core of LHRH-related compound and gelatin surrounded by a
26 shell of D,L-lactic and glycolic acids copolymer. These microparticles are
27 suspended in a diluent for injection either subcutaneously or intramuscularly.
28 [21, 26] These products must be stored at room temperature or lower.
29 Aqueous formulations of LHRH-related compounds are known to exhibit both
30 chemical and physical instability, as well as degradation after irradiation. [12-
31 16, 22-25]

1 Formulations which have been shown to be stable (t_{90} about five years)
2 have been very low concentration (25 $\mu\text{g/ml}$) aqueous, buffered (10 mM, ionic
3 strength of 0.15) solutions stored at temperatures no higher than room
4 temperature (25°C). [15]

5 There is a need for stable formulations of peptides.

6

7 **SUMMARY OF THE INVENTION**

8 The present invention provides stable non-aqueous formulations which
9 are solutions of peptide compounds in polar aprotic solvents. In particular,
10 the peptide compounds are formulated at concentrations of at least about
11 10%. These stable formulations may be stored at elevated temperatures
12 (e.g., 37°C) for long periods of time and are especially useful in implantable
13 delivery devices for long term delivery (e.g., 1-12 months or longer) of drug.

14 In one aspect, the invention provides stable non-aqueous formulations
15 of peptide compounds, said formulations comprising at least one peptide
16 compound in at least one polar aprotic solvent. In a preferred embodiment,
17 the formulation comprises at least about 10% (w/w) peptide compound.

18 In another aspect, the invention provides methods for preparing a
19 stable non-aqueous formulation of a peptide compound, said methods
20 comprising dissolving at least one peptide compound in at least one polar
21 aprotic solvent. Preferred formulations comprise at least about 10% (w/w)
22 peptide compound.

23 In yet a further aspect, the invention provides methods for treating a
24 subject suffering from a condition which may be alleviated by administration
25 of a peptide compound, said methods comprising administering to said
26 subject an effective amount of a stable non-aqueous formulation comprising
27 at least one peptide compound in at least one polar aprotic solvent.

28

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the stability of 40% leuprolide acetate solution (w/w) in dimethylsulfoxide (methylsulfoxide or DMSO) after two months at 80°C as measured by reverse phase HPLC (RP-HPLC).

Figure 2 shows the same sample as Figure 1 injected by size exclusion chromatography (SEC). This figure shows that there is very little aggregation, and what aggregation there is is comprised of dimer and trimer products, with no higher order aggregation.

Figure 3 presents the Arrhenius plot showing the loss of leuprolide from 40% solutions of leuprolide acetate in dimethylsulfoxide (DMSO).

Figure 4 illustrates the chemical and physical stability of a 40% leuprolide solution in DMSO after six months at 80°C.

Figure 5 illustrates the loss of leuprolide from a 40% leuprolide acetate solution in DMSO over a period of six months at 37°C, 50°C, 65°C or 80°C.

Figure 6 illustrates the chemical stability of a 40% leuprolide acetate solution in DMSO over a period of nine months at 37°C.

Figure 7 illustrates that increasing the concentration of the peptide leuprolide in DMSO solution increased stability at 80°C.

Figure 8 illustrates that increasing the moisture content of 40% leuprolide-DMSO formulations resulted in decreased stability at 80°C.

Figure 9 illustrates that, in the formulations shown in Figure 8, chemical degradation products increased with increasing moisture.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is drawn to the unexpected discovery that dissolving peptide compounds in non-aqueous polar aprotic solvents results in stable formulations. Previously known formulations of peptide compounds, which are dilute buffered aqueous solutions containing excipients such as EDTA or ascorbic acid which must be stored at low temperatures (4-25°C), form degradation products using degradation pathways such as acid/base catalyzed hydrolysis, deamidation, racemization and oxidation. In contrast, the presently claimed formulations stabilize peptide compounds at elevated

1 temperatures (e.g., 37°C to 80°C) and at high concentrations (i.e., at least
2 about 10%).

3 Standard peptide and protein formulations consist of dilute aqueous
4 solutions. Drug stability is usually achieved by varying one or more of the
5 following: pH, buffer type, ionic strength, excipients (EDTA, ascorbic acid,
6 etc). For these formulations, degradation pathways requiring water
7 (hydrolysis, deamidation, racemization) cannot be fully stabilized. In contrast,
8 in the present invention, peptides formulated in non-aqueous solutions, such
9 as dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), were shown to
10 be chemically and physically more stable than those formulated in water.
11 DMSO and DMF are considered polar aprotic solvents. Aprotic solvents
12 would be expected to decrease the rate of degradation since they lack the
13 ability to contribute protons to degradation reactions. Conversely, solvents
14 that are more polar than water (for example, the dipole moment of water is
15 1.85, for DMF is 3.82, and for DMSO is 3.96) would be expected to increase
16 the rate of degradation since they can assist in stabilizing the rate determining
17 step and increasing the rate of degradation. However, we discovered that the
18 overall effect of polar aprotic solvents was generally to stabilize solutions of
19 peptides.

20 The invention consists of using non-aqueous, aprotic solvents such as
21 DMSO or DMF to stabilize peptide formulations against both chemical and
22 physical degradation. The discovery consists of the realization that use of
23 DMSO or DMF improves the overall stability of peptides in a wide range of
24 formulation conditions, including high concentrations and elevated
25 temperatures, thus making possible the delivery of peptides in long term
26 implantable devices that would not otherwise be feasible.

27

28 A. Definitions:

29 As used herein, the following terms have the following meanings:

30 The term "chemical stability" means that an acceptable percentage of
31 degradation products produced by chemical pathways such as oxidation or
32 hydrolysis is formed. In particular, a formulation is considered chemically

1 stable if no more than about 20% breakdown products are formed after two
2 months at 37°C.

3 The term "physical stability" means that an acceptable percentage of
4 aggregates (e.g., dimers, trimers and larger forms) is formed. In particular, a
5 formulation is considered physically stable if no more than about 15%
6 aggregates are formed after two months at 37°C.

7 The term "stable formulation" means that at least about 65%
8 chemically and physically stable peptide compound remains after two months
9 at 37°C (or equivalent conditions at an elevated temperature). Particularly
10 preferred formulations are those which retain at least about 80% chemically
11 and physically stable peptide under these conditions. Especially preferred
12 stable formulations are those which do not exhibit degradation after sterilizing
13 irradiation (e.g., gamma, beta or electron beam).

14 The terms "peptide" and/or "peptide compound" mean polymers of up
15 to about 50 amino acid residues bound together by amide (CONH) linkages.
16 Analogs, derivatives, agonists, antagonists and pharmaceutically acceptable
17 salts of any of these are included in these terms. The terms also include
18 peptides and/or peptide compounds which have D-amino acids, modified,
19 derivatized or non-naturally occurring amino acids in the D- or L- configuration
20 and/or peptomimetic units as part of their structure.

21 The term "LHRH-related compound" means luteinizing hormone
22 releasing hormone (LHRH) and its analogs and pharmaceutically acceptable
23 salts. Octa-, nona- and decapeptide LHRH agonists and antagonists are
24 included in the term LHRH-related compounds, as is native LHRH.

25 Particularly preferred LHRH-related compounds include LHRH, leuprolide,
26 goserelin, nafarelin, and other known active agonists and antagonists. [1-21]

27 The term "high concentration" means at least about 10% (w/w) and up
28 to the maximum solubility of the particular peptide.

29 The term "excipient" means a more or less inert substance in a
30 formulation which is added as a diluent or vehicle or to give form or
31 consistency. Excipients are distinguished from solvents such as EtOH, which
32 are used to dissolve drugs in formulations, and from non-ionic surfactants

1 such as Tween 20, which are used to solubilize drugs in formulations, and
2 from preservatives such as benzyl alcohols or methyl or propyl parabens,
3 which are used to prevent or inhibit microbial growth.

4 The term "polar aprotic solvent" means a polar solvent which does not
5 contain acidic hydrogen and does not act as a hydrogen bond donor.

6 Examples of polar aprotic solvents are dimethylsulfoxide (DMSO),
7 dimethylformamide (DMF), hexamethylphosphorotriamide (HMPT), and n-
8 methyl pyrrolidone.

9 The term "non-aqueous protic solvent" means a non-polar solvent
10 which contains hydrogen attached to oxygen or nitrogen so that it is able to
11 form hydrogen bonds or donate a proton. Examples of apolar protic solvents
12 are polyethylene glycols (PEGs), propylene glycol (PG), polyvinylpyrrolidone
13 (PVP), methoxypropylene glycol (MPEG), glycerol and glycofurool.

14

15 B. Preparation of Formulations:

16 The present invention is drawn to non-aqueous formulations of
17 peptide compounds in polar aprotic solvent which are stable for prolonged
18 periods of time at elevated temperatures. Standard dilute aqueous peptide
19 and protein formulations require manipulation of buffer type, ionic strength,
20 pH and excipients (e.g., EDTA and ascorbic acid) to achieve stability. In
21 contrast, the claimed formulations achieve stabilization of peptide compounds
22 by the use of non-aqueous polar aprotic solvents. In particular, stability of
23 high concentrations (at least about 10%, w/w) of compound has been
24 provided by the formulations of the present invention.

25 Examples of peptides and peptide compounds which may be
26 formulated using the present invention include those peptides which have
27 biological activity or which may be used to treat a disease or other
28 pathological condition. They include, but are not limited to
29 adrenocorticotrophic hormone, angiotensin I and II, atrial natriuretic peptide,
30 bombesin, bradykinin, calcitonin, cerebellin, dynorphin A, alpha and beta
31 endorphin, endothelin, enkephalin, epidermal growth factor, fertirelin, follicular
32 gonadotropin releasing peptide, galanin, glucagon, gonadorelin,

1 gonadotropin, goserelin, growth hormone releasing peptide, histrelin, insulin,
2 leuprolide, LHRH, motilin, nafarelin, neurotensin, oxytocin, somatostatin,
3 substance P, tumor necrosis factor, triptorelin, and vasopressin. Analogs,
4 derivatives, antagonists, agonists and pharmaceutically acceptable salts of
5 the above may also be used.

6 The peptide compounds useful in the formulations and methods of the
7 present invention can be used in the form of a salt, preferably a
8 pharmaceutically acceptable salt. Useful salts are known to those of skill in
9 the art and include salts with inorganic acids, organic acids, inorganic bases
10 or organic bases. Preferred salts are acetate salts.

11 Peptides and peptide compounds which are readily soluble in non-
12 aqueous polar aprotic solvents are preferred for use in the present invention.
13 One of skill in the art can easily determine which compounds will be useful on
14 the basis of their solubility, i.e., the compound must be soluble in the
15 particular non-aqueous polar aprotic solvent to at least an acceptable
16 amount. Preferred solubilities are at least about 10% (w/w). Particularly
17 preferred peptide compounds are LHRH-related compounds, including
18 leuprolide and leuprolide acetate.

19 The proportion of peptide may vary depending on the compound, the
20 condition to be treated, the solubility of the compound, the expected dose and
21 the duration of administration. (See, for example, The Pharmacological Basis
22 of Therapeutics, Gilman et al., 7th ed. (1985) and Pharmaceutical Sciences,
23 Remington, 18th ed. (1990), the disclosures of which are incorporated herein
24 by reference.) The concentration of peptide in high concentration
25 formulations may range from at least about 10% (w/w) to the maximum
26 solubility of the compound. A preferred range is from about 20 to about 60%
27 (w/w). The currently more preferred range is from about 30 to about 50%
28 (w/w) and a most preferred range is about 35 to about 45% (w/w).

29 It has unexpectedly been found that increasing the concentration of
30 peptide that is dissolved in the non-aqueous polar aprotic solvent may
31 increase the stability of the peptide formulation. For example, as seen in
32 Figure 7, when solutions of 5, 10, 20 and 40% leuprolide in DMSO were

1 stored for 8 weeks at 80°C with samples taken periodically and analyzed to
2 determine the percentage of leuprolide remaining, formulations containing
3 higher concentrations of leuprolide were more stable than formulations with
4 lower concentrations of leuprolide.

5 Generally, the stable formulations of the present invention may be
6 prepared by simply dissolving the desired amount, which may be a
7 therapeutically effective amount, of the desired peptide compound in the
8 selected non-aqueous polar aprotic solvent. Preferred polar aprotic solvents
9 include DMSO and DMF.

10 Increasing the water contained in the peptide formulations of the
11 present invention increased peptide degradation as shown in Figure 8. It
12 appears that this increase may be due mainly to increasing chemical
13 degradation products, with aggregation remaining relatively constant
14 (Figure 9).

15 It has also been found that non-aqueous protic solvents such as PEG,
16 PG and PVP may optionally be added to the claimed formulations.

17

18 C. Methodology:

19 We have found that stable non-aqueous formulations of peptide
20 compounds may be prepared by dissolving the peptide compound to be
21 formulated in non-aqueous polar aprotic solvents.

22 We have tested these peptide compound formulations, specifically
23 formulations of the LHRH-related compound leuprolide, for stability by
24 subjecting them to accelerated aging at elevated temperature and measuring
25 the chemical and physical stability of the formulations. Results of these
26 studies (shown, for example, in Table II and Figures 1, 2, 4 and 6)
27 demonstrate that these formulations were stable at conditions that
28 approximate or exceed storage for one year at 37°C.

29 We have also tested peptide compound formulations prepared as
30 described herein for stability after 2.5 megarad gamma irradiation. Results,
31 shown in Table III, show that these formulations remained chemically and
32 physically stable after such irradiation.

1 As shown in Table I, we have tested a wide variety of peptide
2 formulations, specifically leuprolide, goserelin, LHRH, angiotensin I,
3 bradykinin, calcitonin, enkephalin, insulin, neurotensin, substance P,
4 trypsinogen and vasopressin, for stability by dissolving (or attempting to
5 dissolve) them in the non-aqueous polar aprotic solvent DMSO, then
6 subjecting them to accelerated aging at elevated temperatures. The stability
7 of the formulations was measured. Results are presented in Table I as half-
8 life at 37°C assuming an $E_a = 22.2$ kcal/mole. A wide range of the peptides
9 tested were soluble in DMSO and remained stable under the test conditions.
10 The solubility of a particular peptide in a particular non-aqueous polar aprotic
11 solvent and the stability of the resulting solution are easily determined using
12 routine procedures known to those of ordinary skill in the art.

13

Table I: Stability of Peptides Formulated in DMSO

FORMULATION	HALF-LIFE* (Temperature)
40% Leuprolide	29.8 years (37°C)
40% Goserelin	5.0 years (80°C)
20% LHRH	8.2 years (65°C)
20% Angiotensin I	4.2 years (65°C)
5% Angiotensin I	4.1 months (50°C)
20% Bradykinin	2.9 months (65°C)
40% Calcitonin	insoluble (80°C)
20% Calcitonin	2.4 months (80°C)
5% Calcitonin	100% stability at 2 months (50°C)
10% Enkephalin	1.9 months (80°C)
5% Enkephalin	2.6 months (50°C)
20% Insulin	insoluble gel (65°C)
5% Neurotensin	5.0 months (50°C)
5% Substance P	3.0 months (50°C)
40% Trypsinogen	insoluble crystal/gel (65°C/80°C)
20% Trypsinogen	insoluble gel (65°C)
5% Trypsinogen	5.9 months (50°C)
40% Vasopressin	degraded (80°C)
20% Vasopressin	11.8 days (65°C)
*Half-life at 37°C assuming $E_a = 22.2$ kcal/mole.	

Formulations of 40% peptide in DMSO stored for six months at 37°C, 50°C, 65°C and 80°C showed non-linear Arrhenius kinetics as measured by overall loss of peptide from the solution, showing stability of these formulations at elevated temperatures. Analysis of data collected at 37°C gave a t_{90} of 14.4 months, indicating that stability at 37°C is still very good.

1 Temperature appears to affect both the rate of degradation and the
2 ratio of the degradation products of the formulations of the present invention.
3 Studies of leuprolide-DMSO formulations have shown that at 65°C and 80°C
4 oxidation appears to be the major chemical degradation pathway.
5 Conversely, at 37°C and 50°C hydrolysis and isomerization appear to be the
6 predominant degradation routes for these formulations.

7 We have also unexpectedly found that certain peptide formulations of
8 the present invention are bacteriostatic (i.e., inhibit bacterial growth),
9 bactericidal (i.e., cause the death of bacteria), and sporicidal (i.e., kill spores).
10 In particular, leuprolide formulations of 50-400 mg/ml exhibited bacteriostatic,
11 bactericidal and sporicidal activity. The stability of the samples was
12 unaffected by spiking with bacteria, indicating that the enzymes released from
13 the killed and lysed bacteria did not adversely affect the stability of the
14 product. This demonstrates that these formulations were not conducive to
15 enzymatic activity.

16 Some peptides, for example calcitonin and leuprolide, are known to be
17 physically unstable, exhibiting aggregation, gelation and fibrillation when
18 formulated in aqueous solution. Improving physical stability can increase
19 bioavailability, alleviate sensitization and immune response, and allow for
20 easier parenteral administration, including administration using implantable
21 drug delivery systems.

22 It has unexpectedly been found that certain peptides, such as
23 leuprolide, goserelin and calcitonin, formulated in the non-aqueous polar
24 aprotic solvents of the present invention do not gel. No gelation was found
25 even after 12 months at 37°C. This is apparently because non-aqueous polar
26 aprotic solvents cause peptides to form a random coil/alpha helix
27 conformation that does not refold into a beta sheet structure and, therefore,
28 does not gel. Thus, these solvents have an anti-gellant effect.

29 A major aspect of the invention is that non-aqueous solutions
30 containing peptide compounds in polar aprotic solvents are chemically and
31 physically stable at high temperatures for long periods of time. Such
32 formulations are stable even when high concentrations are used. Thus, these

1 formulations are advantageous in that they may be shipped and stored at
2 temperatures at or above room temperature for long periods of time. They
3 are also suitable for use in implantable delivery devices.

4 5 DISCLOSURE OF EXAMPLES OF THE INVENTION

6 The following methods were used to perform the studies in the
7 Examples that follow.

8 9 1. Preparing leuprolide acetate solutions

10 Leuprolide acetate (obtained, for example, from Mallinckrodt, St. Louis,
11 Missouri) was weighed and dissolved with stirring or centrifugation in vehicle
12 (DMSO, DMF, DMSO/PEG, DMSO/PG, or DMSO/PVP) at the appropriate
13 concentration. The term dry DMSO refers to DMSO formulations prepared in
14 a low moisture environment (i.e., dry N₂ atmosphere).

15 Unless otherwise noted, leuprolide free base content was calculated
16 from certificate of analysis potency values to be 37°C free base. This was
17 40% leuprolide acetate, except as noted.

18 19 2. Preparation of reservoirs

20 The reservoirs of implantable drug delivery devices (as disclosed in
21 U.S. Patent Application Serial No. 08/595,761, incorporated herein by
22 reference) were filled with the appropriate leuprolide acetate solution. The
23 formulation was filled into titanium or polymer reservoirs with a polymer plug
24 blocking each end. The filled reservoir was then sealed in a polyfoil bag and
25 placed in a stability testing oven.

26 It should be noted that the formulations in the reservoirs of these
27 devices are completely isolated from the outside environment.

3. Reverse Phase-HPLC (RP-HPLC)

All stability samples were analyzed for leuprolide concentration and % peak area using a gradient elution reversed-phase HPLC assay with a refrigerated autosampler (4°C) to minimize sample degradation. The chromatographic conditions used are listed below.

RP-HPLC Chromatographic Conditions

Description	Parameter									
Column	HaiSil C18, 4.6 X 250mm, S/N 5103051									
Flow Rate	0.8 mL min ⁻¹									
Injection Volume	20 µL									
Detection	210 nm									
Leuprolide Retention Time	Between 25-30 minutes									
Mobile Phase	A = 100 mM Sodium Phosphate, pH 3.0 B = 90% Acetonitrile/Water									
Gradient	Minutes	0	5	25	40	41	46	46.1	50	
	%B	15	26.5	26.5	65	85	85	15	15	

Leuprolide standards (in water) at 4 to 6 different concentration levels, typically between 0.1 - 1.2 mg/mL, were run along with the stability samples. The stability samples were bracketed by the standard sets, with no more than 40 samples in between the standard sets. All peaks between the void volume and 45 minutes of the run were integrated. The integrated peak areas for the leuprolide standards were plotted as a function of the concentration. The leuprolide concentrations for the stability samples were then calculated using linear regression. The % peak areas for the leuprolide peak, the sum of all the peaks eluting before leuprolide (labeled "others"), and the sum of all the peaks eluting after leuprolide (labeled "aggregates") were also recorded and plotted as a function of the sample timepoints.

4. Size Exclusion Chromatography (SEC)

Selected stability samples were analyzed for % peak area and molecular weights using an isocratic solution SEC assay with a refrigerated autosampler (4°C). The chromatographic conditions used are listed below.

SEC Chromatographic Conditions

Description	Parameter
Column	Pharmacia Peptide, HR 10/30, 10 X 300 mm
Flow Rate	0.5 mL min ⁻¹
Injection Volume	20 µL
Detection	210 nm
Leuprolide Retention Time	Approximately 25 minutes
Mobile Phase	100 mM Ammonium Phosphate, pH 2.0, 200 mM Sodium Chloride, 30% Acetonitrile

The void volume and total volume for the size exclusion column was needed for the calculation of the molecular weights. The BioRad high molecular weight standard and 0.1% acetone were used to determine the void volume and total volume respectively. The retention times for the first peak in the BioRad standard and the acetone peak were recorded and converted to volume units using the equations below. Since these values are constant for a particular SEC column and HPLC system, the void and total volumes were redetermined whenever changes to the SEC column or HPLC system were made. A standard run was then made followed by the stability samples. The standard mixture contained approximately 0.2 mg/mL of the following peptides: Bursin (MW=449), WLFR peptide (MW=619), Angiotensin (MW=1181), GRF (MW=5108), and Cytochrome C (MW=12394). These standards were chosen because they bracketed leuprolide molecular weight and all had basic pI (9.8 - 11.0), similar to leuprolide.

1 The % peak areas were recorded for all the peaks. The molecular
2 weights for the species separated were calculated using the equations below.

3 $V_s = \text{flow rate (mL/min)} \times \text{sample peak retention time (min)}$

4 $V_o = \text{flow rate (mL/min)} \times \text{void volume peak retention time (min)}$

5 $V_t = \text{flow rate (mL/min)} \times \text{total volume peak retention time (min)}$

6
7
$$K_d = \frac{V_s - V_o}{V_t - V_o}$$

8
9

10 Where:

11 V_s = standard or sample volume

12 V_o = void volume

13 V_t = total volume

14
15 V_s was calculated to each peptide standard peak. K_d for each peptide
16 standard was then calculated using the values for V_t and V_o determined
17 earlier. The linear regression line from the plot of $\log MW$ vs. K_d^{-1} was used
18 to determine the molecular weights for each peak in the stability sample. The
19 % peak areas for the stability samples were also recorded.

20
21 5. Instrumentation and Materials

22 The instrumentation and materials used for RP-HPLC and SEC were
23 as follows:

24 Waters Millennium HPLC system consisting of 717 autosampler, 626 pump,
25 6000S controller, 900 photodiode array detector, and 414 refractive
26 index detector (Waters Chromatography, Milford, MA)

27 HPLC vials, for 48-position and 96-position (Waters Chromatography, Milford,
28 MA)

29 HaiSil C18, 120 A, 5 μ m 4.6 x 250 mm HPLC column (Higgins Analytical,
30 Mountain View, CA)

31 Pharmacia Peptide, HR 10/30 SEC column (Pharmacia Biotech, Piscataway,
32 NJ)

1 The following examples are offered to illustrate this invention and are
2 not meant to be construed in any way as limiting the scope of this invention.

3

4 **EXAMPLE 1**

5 Accelerated Stability Studies of Leuprolide Acetate Formulations

6 Formulations of 40% (w/w) leuprolide acetate (equivalent to about 37%
7 leuprolide free base) in vehicle were prepared as described above and used
8 to fill the reservoirs of implantable drug delivery devices, also as described
9 above. All reservoirs were made of titanium.

10 The filled devices were subjected to accelerated aging by storing them
11 at elevated temperatures (80°C) for seven days in an oven (Precision
12 Scientific or Thelco). This is equivalent to about 1.5 years at 37°C or about
13 four years at room temperature (25°C).

14 The samples were analyzed using RP-HPLC and SEC as described
15 above to determine the chemical and physical stability of the aged
16 formulations.

17 Results, presented in Table II, demonstrate that these formulations
18 were able to maintain the stability of the LHRH-related compound leuprolide.
19 In each case, at least 65% leuprolide was retained.

Table II

**Stability of Leuprolide Acetate Polar Aprotic Formulations After 7 Days
at 80°C in Titanium Reservoirs**

Formulation	% Leuprolide at Day 7
40% in DMSO	92
40% in DMSO/PEG (50/50)	90
40% in DMSO/PG (50/50)	86
40% in DMSO/PVP (50/50)	93
40% in DMF	91
40% in dry DMSO	89

EXAMPLE 2**Stability Studies of Irradiated Leuprolide Acetate Formulations**

Formulations of 40% (w/w) leuprolide acetate in DMSO were prepared as described above and used to fill the reservoirs of drug delivery devices, also as described above. All reservoirs were made of titanium.

The filled devices were sent to Sterigenics (Tustin, California) where they were subjected to 2.5 megarad gamma irradiation using Cobalt 60, 3-level "tote box" irradiation in Sterigenics' Tustin Main Cell. In Table III, the samples labeled "cold" were shipped and irradiated on dry ice. Samples were then subjected to accelerated aging as in Example 1. Samples were taken at day 0 and day 7, and analyzed using RP-HPLC and SEC as described above to determine the chemical and physical stability of the irradiated formulations.

Results, presented in Table III, demonstrate that these leuprolide acetate formulations were stable after irradiation. In every case, at least 65% leuprolide was retained, with low levels of aggregate formation.

Table III

Stability of 40% (w/w) Leuprolide Acetate Polar Aprotic Formulations After 2.5 Megarad Gamma Irradiation in Titanium Reservoirs

Formulation	Irradiation	% Leuprolide at Day 7 (RP-HPLC)	SEC					
			Day 0			Day 7		
			% monomer	% dimer/trimer		% monomer	% dimer/trimer	
40% in DMSO	Yes	100	98.1	0.5		97.7	1.9	
40% in DMSO	No	90	100	0		98.5	1.1	
40% in DMSO	Cold	99	99.1	0.2		98.3	1.4	
40% in DMSO	Yes	95	99.1	0.8		95.3	2	
40% in DMSO	No	N.D.	100	0		106.1	0	
40% in DMSO	Yes	90	99.4	0.6		99.4	2.2	
40% in DMSO	No	100	100	0		104	1	
40% in DMSO	Yes	88	99.5	0.5		97.7	1.8	
40% in DMSO	Yes	83	99.5	0.5		91.7	1.5	

EXAMPLE 3**Accelerated Long-Term Stability Studies of Leuprolide Acetate Formulations**

Solutions of 40 % leuprolide acetate (w/w) in DMSO were prepared, loaded into reservoirs, stored for two months at 80°C and analyzed as described above. Results, shown in Figures 1 (RP-HPLC) and 2 (SEC) show that 81.1% leuprolide was recovered, with only 14.6% chemical degradation and 5.1% physical aggregation.

Leuprolide acetate solutions were prepared, loaded, stored at 80°C for six months and analyzed as described above. Figure 4 is a plot of leuprolide, and its chemical and physical degradation products recovered over the six month time period, showing that we accounted for all the peptide material we started with and that these formulations showed good stability at 80°C. The sum of these three elements is also presented as mass balance. Figure 5 is a plot of the natural log of these data, showing that these formulations exhibited linear kinetics over the entire temperature range tested.

The chemical stability of 40% leuprolide acetate solutions prepared and analyzed as described above is presented in Figure 6. After nine months at 37°C more than 90% (93.5%) leuprolide was present, with less than 5% (2.9%) chemical degradation products (shown as "early" in the figure) and less than 5% (2.3%) physical degradation products (shown as "late" and based on the RP-HPLC profile, but in good agreement with SEC) being formed.

Solutions of 40% leuprolide acetate (w/w) in DMSO were prepared, loaded into reservoirs, stored at 37°C, 50°C, 65°C or 80°C and analyzed using RP-HPLC as described above. Results were calculated as described in Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 3rd ed., Martin et al., Chapter 14 (1983) and showed that loss of leuprolide from DMSO formulations was non-linear. The data are shown below and an Arrhenius plot is presented in Figure 3.

- 5 Because Arrhenius plots of DMSO formulations stored at 80°C showed that loss of leuprolide was non-linear, stability data collected at 37°C was used to calculate a t_{90} for these formulations of 14.4 months at 37°C.

DMSO		
°C	Kobs (months ⁻¹)	t _{1/2} (months)
37	7.29×10^{-3}	95.1
50	9.74×10^{-3}	71.2
65	2.48×10^{-2}	27.9
80	0.108	6.4

E_a = non-linear

10

EXAMPLE 4

Stability Studies of Leuprolide Acetate Formulations in DMSO/Water

- Formulations of 40% leuprolide acetate (w/w) in DMSO, DMSO/water (95:5, 90:10, 70:30, 50:50, and 30:70) and water were prepared as described
15 above and incubated for seven days at 80°C. Fourier Transfer Infrared Spectroscopy (FTIR) analysis was performed at day 0 and at day 7.

- Results showed that the structural conformation of leuprolide changed very little after this accelerated aging for all the formulations tested. In general, peptide structure was predominantly random coil or α -helix in DMSO
20 formulations, while peptide structure was predominantly β -sheet in water formulations.

- Modification of the above-described modes of carrying out various embodiments of this invention will be apparent to those of skill in the art following the teachings of this invention as set forth herein. The examples
25 described above are not limiting, but are merely exemplary of this invention, the scope of which is defined by the following claims.

5 What is claimed is:

1. A stable non-aqueous formulation of a peptide compound
comprising:

- 10 a) at least one peptide compound; and
b) at least one polar aprotic solvent.

2. The formulation of Claim 1 which comprises at least about 10%
(w/w) peptide compound.

15 3. The formulation of Claim 1 which comprises at least about 30%
(w/w) peptide compound.

4. The formulation of Claim 1 wherein said peptide compound is
an LHRH-related compound.

20 5. The formulation of Claim 4 wherein said peptide compound is
selected from the group consisting of leuprolide, LHRH, nafarelin and
goserelin.

25 6. The formulation of Claim 1 of which is stable at 80°C for at least
2 months.

7. The formulation of Claim 1 which is stable at 37°C for at least 3
months.

30 8. The formulation of Claim 1 which is stable at 37° C for at least
one year.

9. The formulation of Claim 1 which is adapted for use in an
35 implantable drug delivery device.

5

10. The formulation of Claim 1 which further comprises a non-aqueous protic solvent.

11. The formulation of Claim 1 wherein said polar aprotic solvent is
10 selected from the group consisting of DMSO and DMF.

12. The formulation of Claim 1 wherein said polar aprotic solvent provides an anti-gellant effect.

13. The formulation of Claim 1 which consists essentially of about
15 30% to about 50% (w/w) of the LHRH-related compound leuprolide acetate in DMSO.

14. The formulation of Claim 1 which consists essentially of
20 leuprolide and DMSO in the proportions of 370 mg leuprolide in 1 ml DMSO.

15. The formulation of Claim 1 which is stable after irradiation.

16. A method for preparing the stable non-aqueous formulation of
25 Claim 1 comprising dissolving at least one peptide compound in at least one polar aprotic solvent.

17. The method of Claim 16, wherein at least about 10% (w/w) peptide compound is dissolved.

30

18. The method of Claim 16 wherein at least about 30% (w/w) peptide compound is dissolved.

19. The method of Claim 16 wherein said peptide compound is an
35 LHRH-related compound.

5

20. The method of Claim 19 wherein said peptide compound is selected from the group consisting of leuprolide, LHRH, nafarelin and goserelin.

10

21. The method of Claim 16 further comprising the step of adding a non-aqueous protic solvent.

22. The method of Claim 16 wherein about 30% to about 50% (w/w) of the LHRH-related compound leuprolide acetate is dissolved in DMSO.

15

23. The method of Claim 16 wherein 370 mg leuprolide is dissolved in 1 ml DMSO.

20

24. A method for treating a subject suffering from a condition which may be alleviated by administration of a peptide compound comprising administering to said subject an effective amount of the formulation of Claim 1.

25

25. The method of Claim 24 wherein said administration is parenteral administration.

26. The method of Claim 24 wherein said administration is long-term continuous administration.

30

27. The method of Claim 26 wherein said administration is accomplished by use of an implantable drug delivery device.

28. The method of Claim 24 wherein said condition is prostatic cancer and said peptide compound is leuprolide.

35

5 29. The method of Claim 28 wherein at least about 80 micrograms
of leuprolide is administered daily.

 30. The method of Claim 29 wherein said daily administration
continues for a period selected from the group consisting of 3 months,
10 6 months and 12 months.

 31. The method of Claim 30 wherein said daily administration for
said period is continuous administration accomplished using an implantable
drug delivery system.

15

 32. The method of Claim 24 wherein said condition is prostatic
cancer and said peptide compound is an LHRH antagonist.

1/8

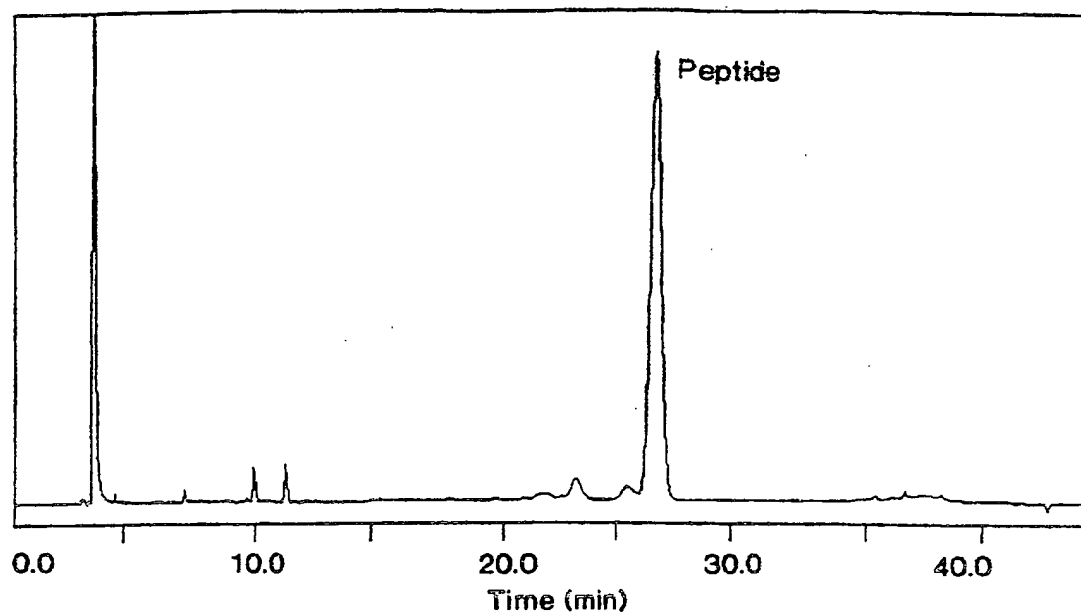


FIG. 1

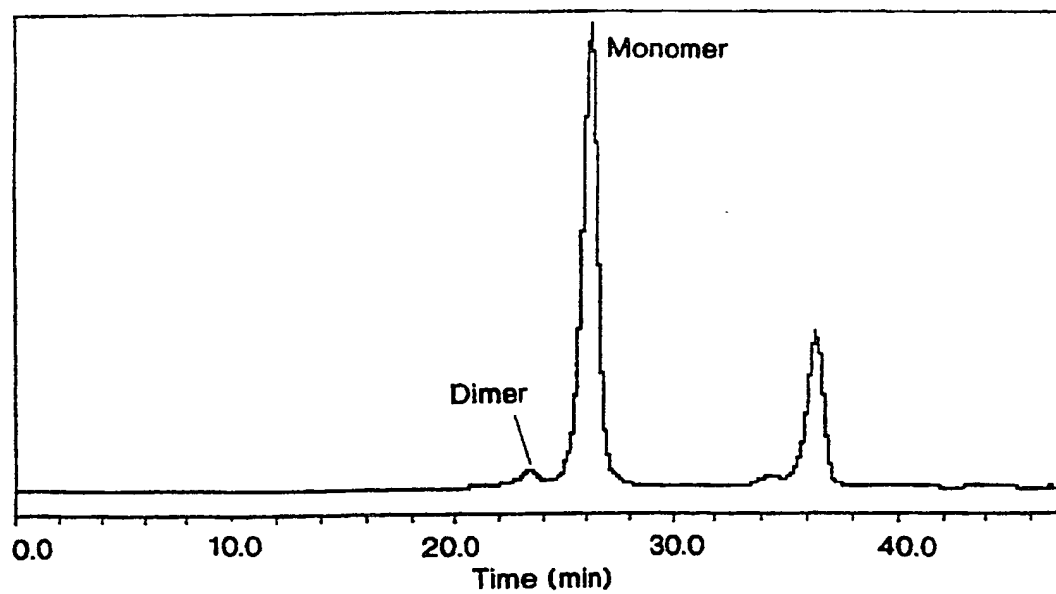


FIG. 2

2 / 8

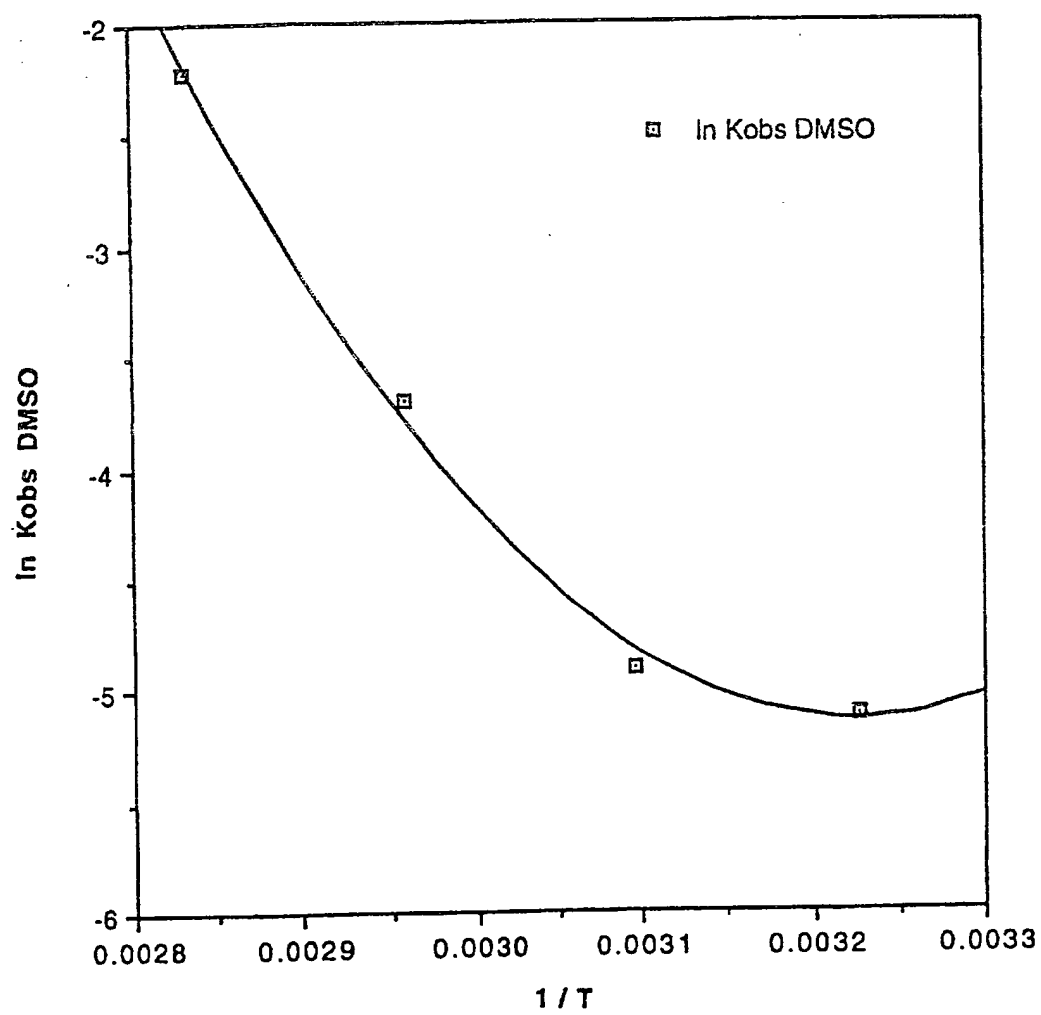


FIG. 3

3/8

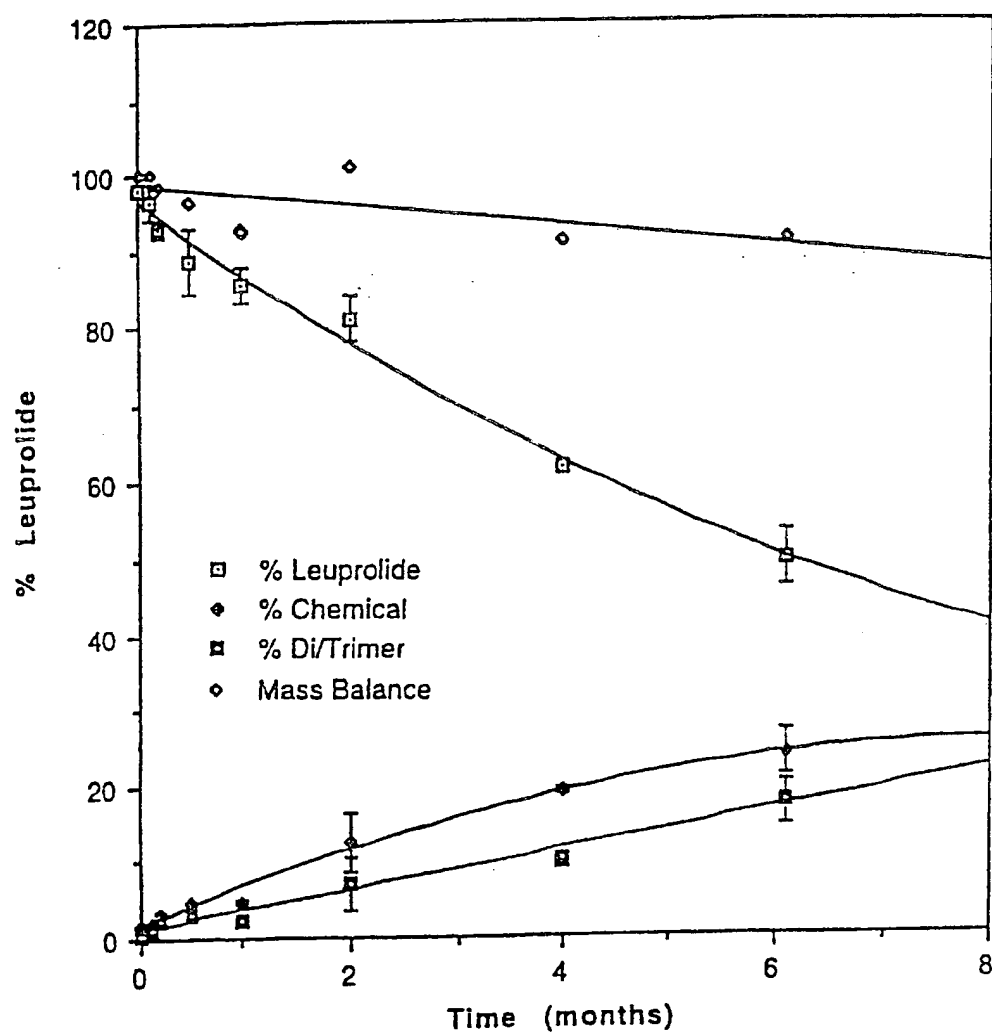


FIG. 4

4 / 8

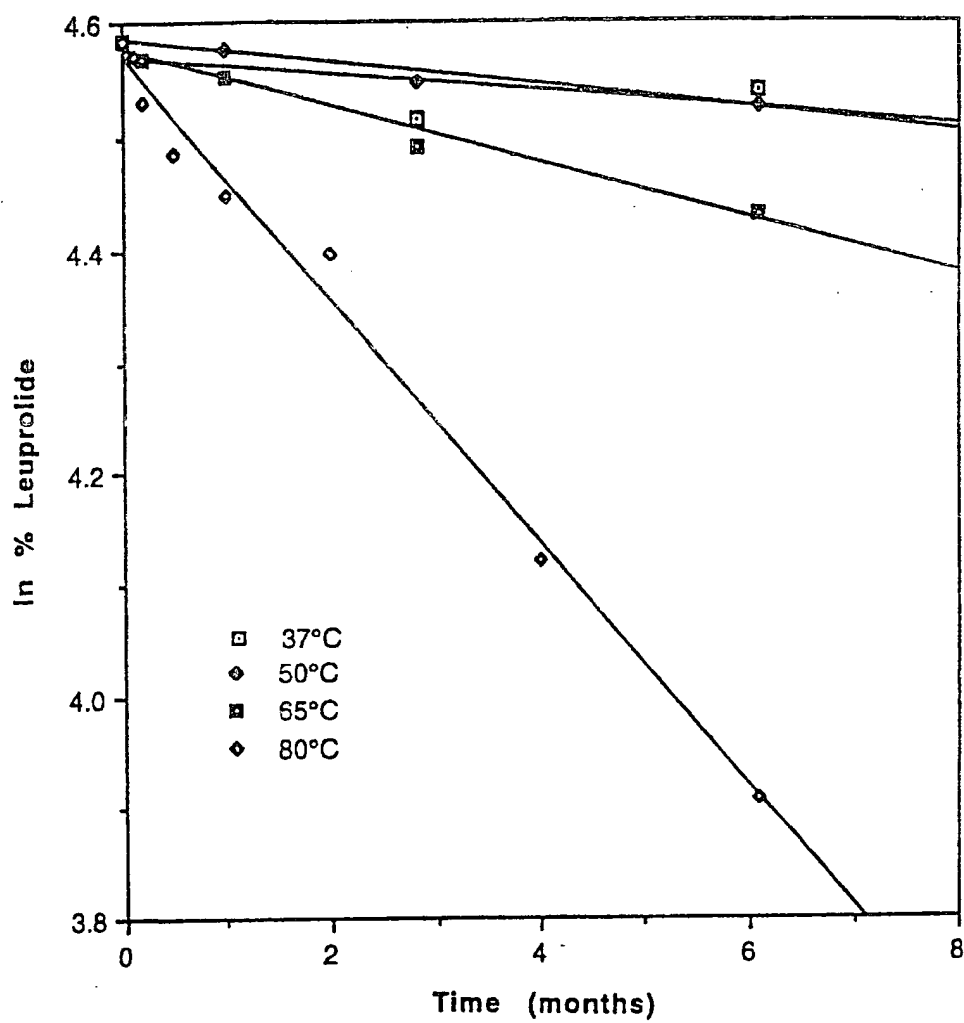


FIG. 5

5 / 8

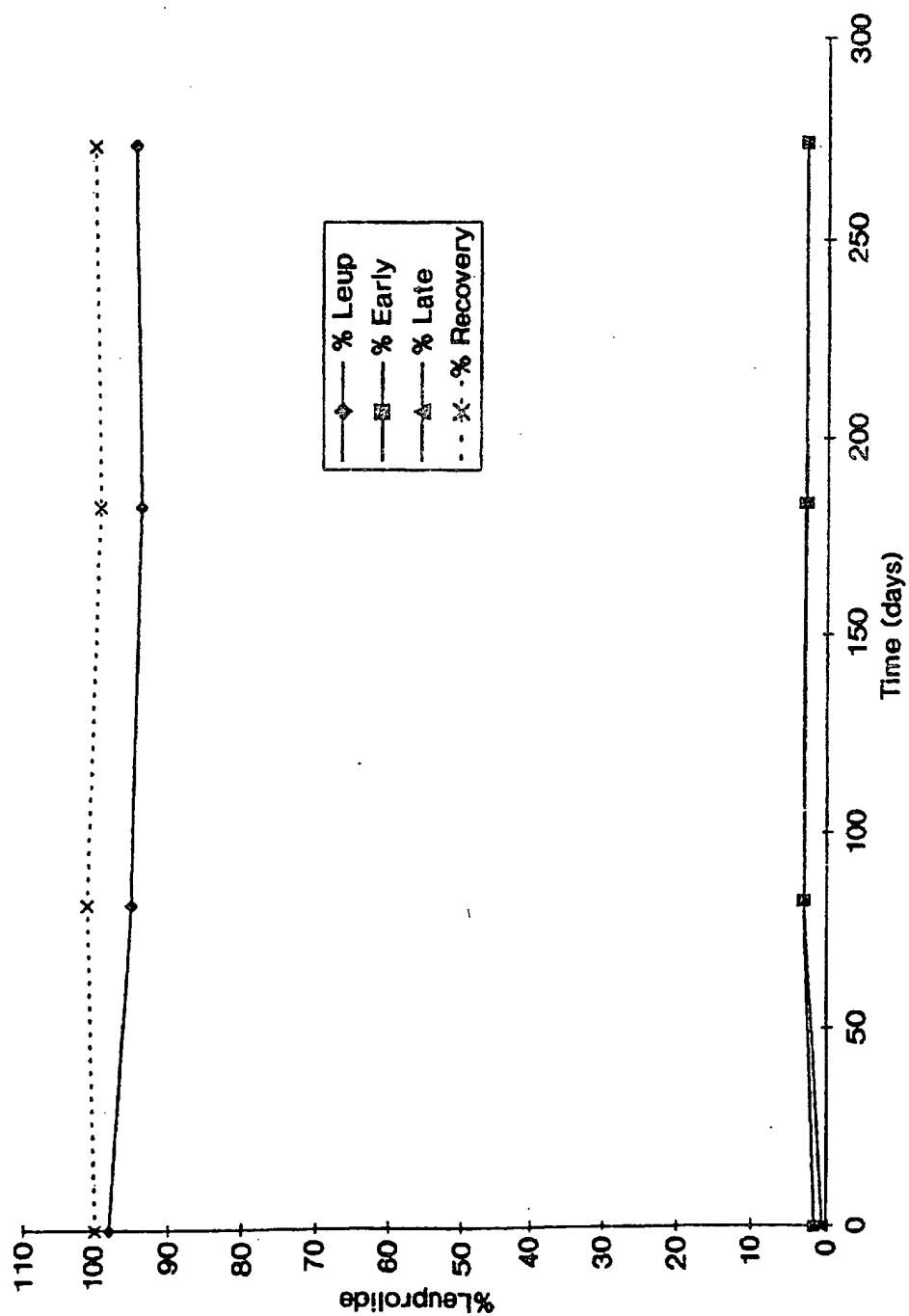


FIG. 6

6 / 8

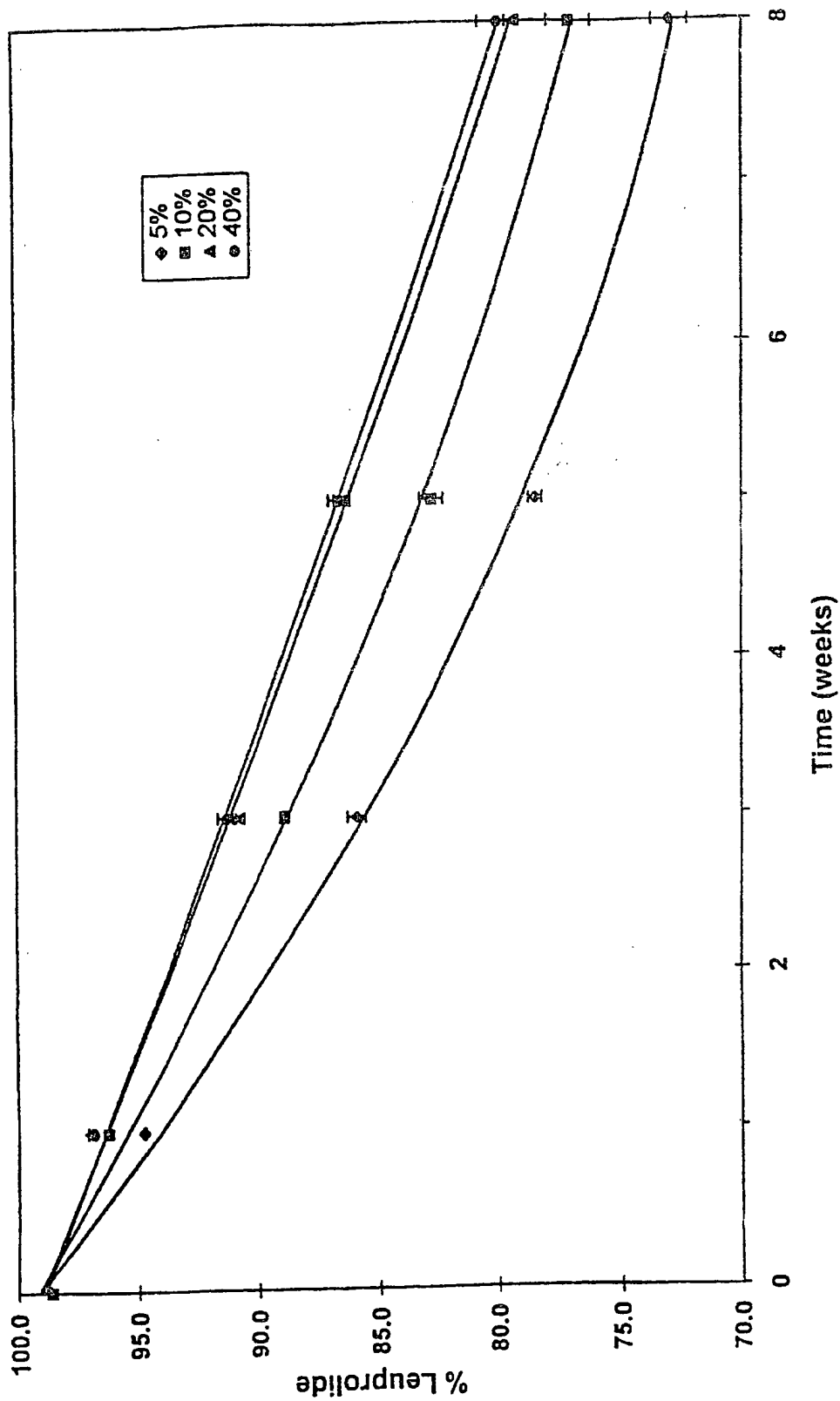


FIG. 7

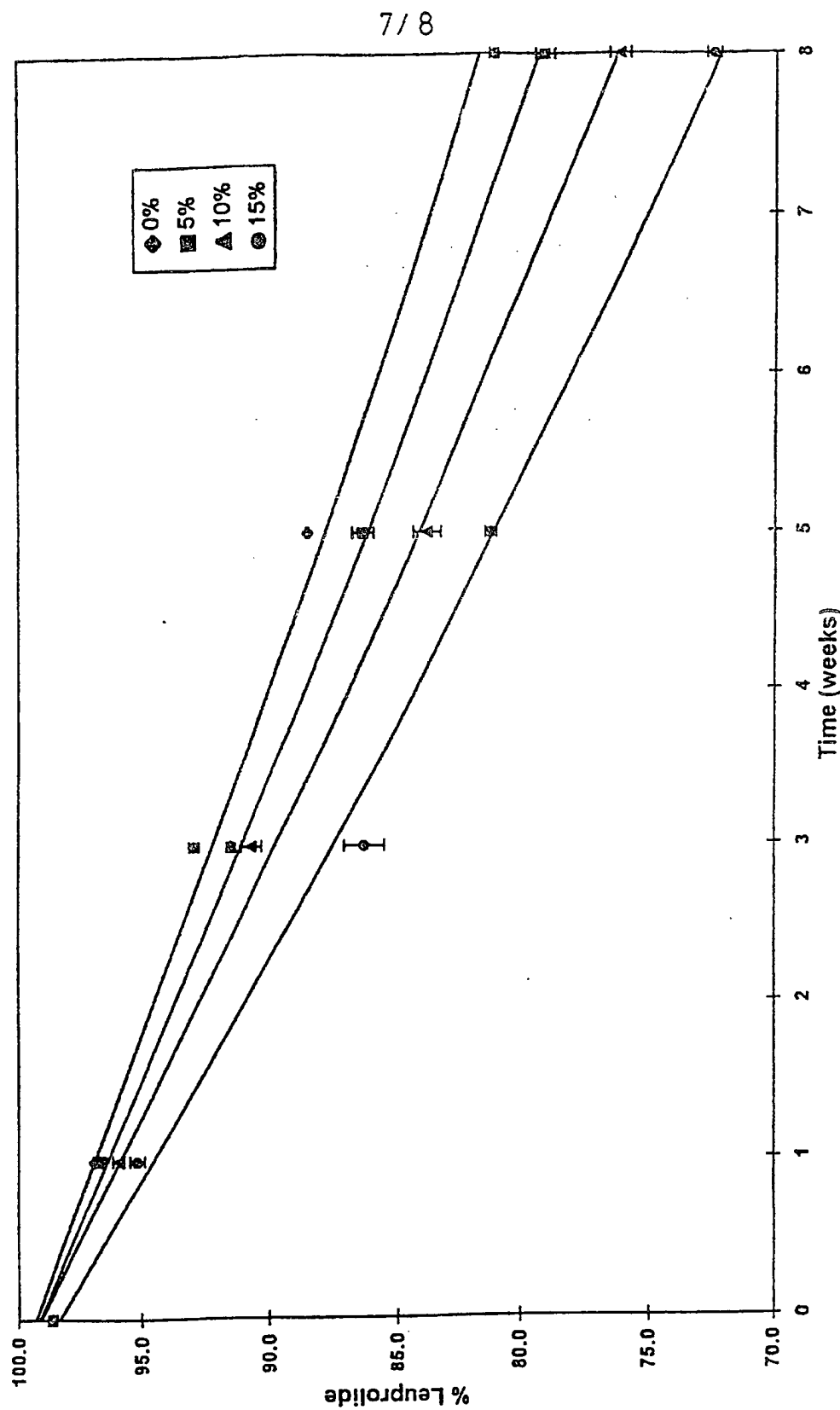
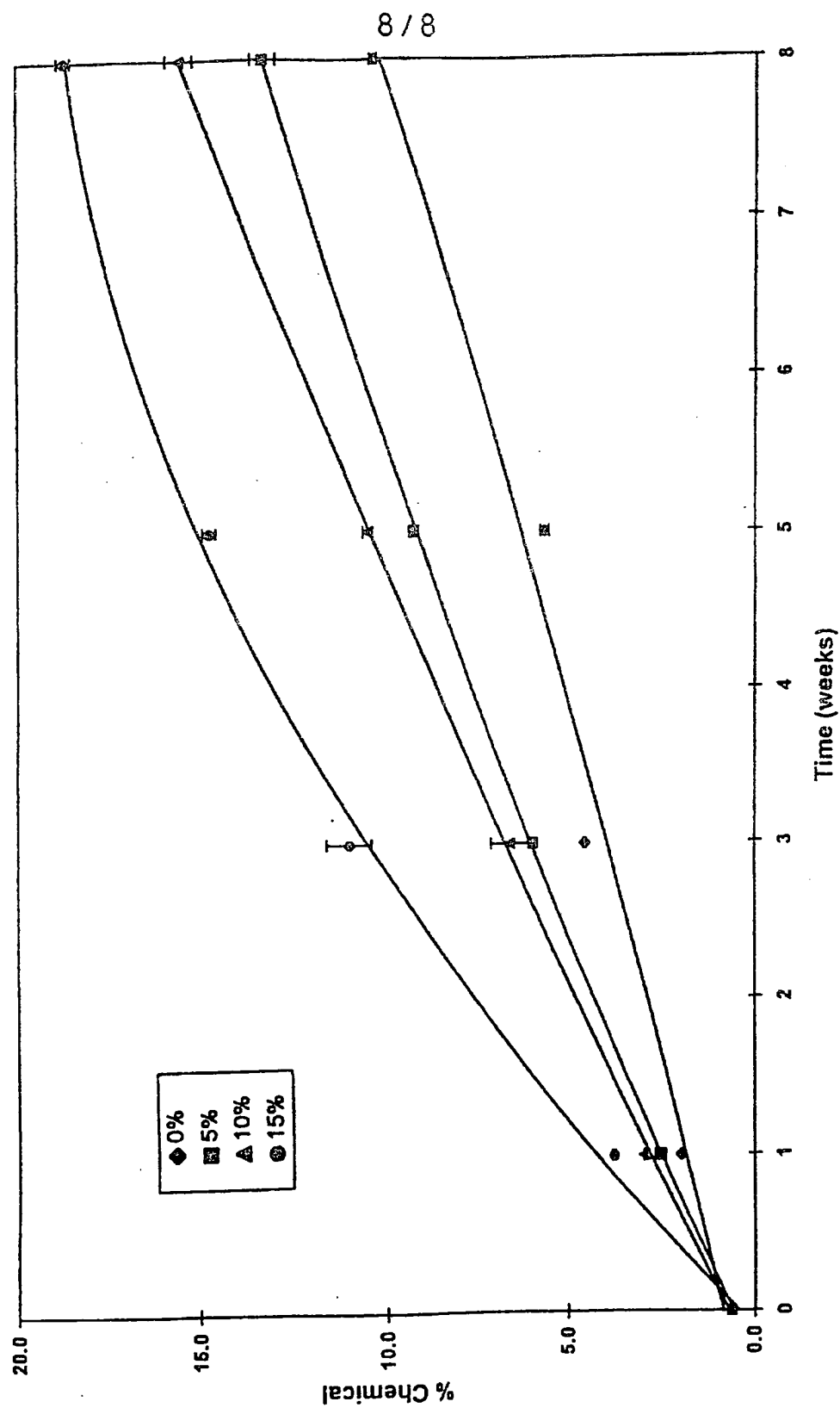


FIG. 8



Time (weeks)

FIG. 9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/11450

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K38/04 A61K38/08 A61K38/09 A61K38/24 A61K47/08 A61K47/16 A61K47/18 A61K47/20		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 119 248 A (MCMULLEN JOHN KENNETH) 16 November 1983	1-3,6-9, 11,15, 16,24-27
Y	see the whole document	10,21
Y	WO 94 19020 A (GENENTECH INC ;CLELAND JEFFREY L (US); JONES ANDREW J S (US)) 1 September 1994 see the whole document	10,21
A	GB 2 008 403 A (CHRISTIE R B PARSONS J A;RUDMAN C G) 6 June 1979	
A	GB 1 098 151 A (CROWN ZELLERBACH CORP.) 10 January 1968	
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
Date of the actual completion of the international search 26 November 1997		Date of mailing of the international search report 11. 12. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Fischer, W

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/11450

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 24-32

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 24-32 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/11450

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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